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A study of the structure of the stomata of two species of Citrus in relation to citrus canker

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(WITH ONE TEXT FIGURE)

There have been many conjectures regarding the causes of differences in resistance of different plants to fungous and bacterial diseases. Usually these differences can not be correlated with any easily recognized characters of the plants in such a way that a definite character may be associated with the disease resistance. The stomatal structure, however, appears to yield such a character in certain of the species of *Citrus* in their relations to citrus canker (*Pseudomonas citri* Hasse).

Marked differences have been observed in the various species and cultivated varieties of *Citrus* in their resistance to canker. Certain of the mandarin varieties, notably "Szinkum," are very resistant, while grapefruit is highly susceptible, as clearly shown by Lee.* It has further been shown by Peltier† and by Lee‡ that all species of *Citrus* and most of the related genera of Rutaceae can be successfully inoculated with canker by pricking the leaves. This seems to indicate that the resistant sorts that can be thus

* Further data on the *Citrus* canker affection of *Citrus* species and varieties at Lamao. Philippine Agr. Rev. **11**: 200–206. 1918.

† Susceptibility and resistance to *Citrus* canker of the wild relatives, *Citrus* fruits and hybrids of the genus *Citrus*. Jour. Agr. Research **14**: 337–357. 1918.

‡ Further data on the susceptibility of rutaceous plants to *Citrus* canker. Jour. Agr. Research **15**: 661–666. 1918.

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inoculated have internal tissues susceptible to canker, but into which canker cannot ordinarily penetrate. Their resistance is believed to be due to differences in the structure of the stomata.*

The present paper gives a comparison of the stomatal structures of two kinds of *Citrus*, one resistant to canker in the manner mentioned above and the other susceptible. For this purpose the Szinkum mandarin (*Citrus nobilis* var. *Szinkum*) was chosen as a highly resistant sort, and a seedling of the Florida grapefruit (*C. grandis*) was chosen as an example of a susceptible sort. These sorts belong to closely related species, they differ comparatively little in leaf morphology, and therefore such differences as are noted in the stomata are the more likely to be directly related to their canker resistance or susceptibility.

PROCEDURE

Young leaves two thirds of their mature size were gathered from the plantation at the College of Agriculture, University of Philippines, on April 10, 1920, and were preserved in alcohol of about 80 per cent concentration. Only the young leaves were used in this study, because the older leaves are no longer susceptible to canker infection in either species, and the old leaves are exceedingly difficult to section for microscopical study. The sectioning and structural study was carried out at the New York Botanical Garden during June, 1920.

Thin slices were cut with a razor parallel to the upper and under surfaces of the leaves. Those from the upper surface showed no stomata. The sections from the under surface were mounted, partly with the cuticle uppermost and partly with the cuticle below. Cross sections were also made of the leaves parallel to their margins. Free-hand sections were found to be the most satisfactory. Imbedding in paraffin was also tried, but the waxy portion was apparently removed from the leaves or rendered transparent by this treatment.

The stomata of the two species were found to be similar in size, general form and mechanism of opening and closing. They are

* The opinion has been expressed that this resistance is due to the epidermis, and evidence in support of this view is given in a paper to be published by Lee and the writer on the resistance of *Citrus nobilis* to *Citrus* canker, with a suggestion for the production of resistant varieties.

both similar to the *Achellea* type described by Copeland.* They differ from this type in having no thickening of the inner half of the ventral wall, and in having no ridge of exit. The general appearance of the stomata of each sort is shown by drawings made under camera lucida (FIG. 1). The stomata were all closed, on account of the treatment with alcohol, except for a few rigid stomata which remained open. The drawings are of closed stomata and therefore represent the minimum widths of aperture.

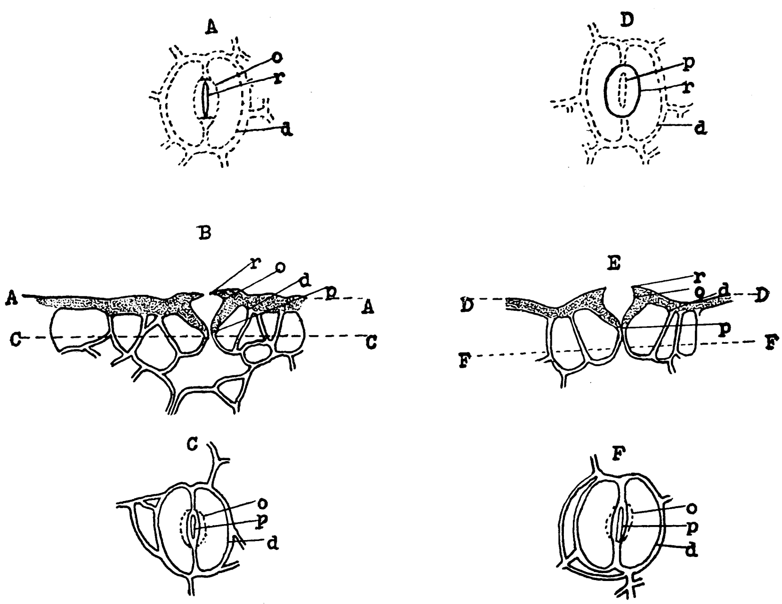

FIG. 1

A, *B* and *C*. Stomata of Szinkum mandarin, × 570: *A*, surface view; *B*, median cross section; *C*, under view. Showing ridge of entrance (*r*); outer chamber (*o*); pore (*p*); and dorsal wall of guard cells (*d*).

D, *E* and *F*. Stomata of Florida seedling grapefruit, × 570, showing same parts as in *A*, *B* and *C*.

The surface views (FIGS. 1, *A*, and 1, *D*) show the ridge of entrance, *r*, to be extremely narrow in the Szinkum mandarin, and to be broadly oval in the Florida seedling grapefruit. This difference was found to be constant and very striking in all of the material examined. The average dimensions of the opening in the cuticle surrounded by the ridge of entrance in the Szinkum

* The mechanism of stomata. Ann. Bot. **16**: 342, *f. 15–17*. 1902.

mandarin were 6 μ in length and 0.6 μ in width. These dimensions were very uniform, the extreme lengths being 5 and 7 μ, and the extreme widths 0.5 and 1.5 μ. Therefore twenty-five measurements were deemed sufficient to give a satisfactory average. Sixty corresponding measurements of Florida seedling grapefruit stomata gave an average length of the opening of 9.8 μ, and a width of 6.6 μ. The extreme lengths were 7 μ and 15 μ, and the extreme widths 5 μ and 11 μ. Thus the narrowest opening in the case of Florida seedling grapefruit (5 μ) was more than three times as wide as the widest in Szinkum mandarin (1.5 μ). By focusing downward, the outlines of the guard cells become visible and are shown at *d* in both Figs. 1, *A*, and 1, *D*. In addition, the outline of the wall of the outer chamber is shown at *o* in 1, *A*, and the outline of the pore, *p*, is shown in 1, *D*.

The parts shown in the median cross sections (FIGS. 1, *B*, and 1, *E*) are labelled to correspond to those described above. The positions of the views shown in 1, *A* and 1, *D*, are indicated by the horizontal lines *AA* and *DD*, and the positions of the FIGS. 1, *C*, and 1, *F*, are shown in a similar manner. The shaded portions of these drawings show the portions of the cell walls which are cutinized. The most prominent differences between the two species is again seen to be the ridge of entrance, *r*, which is elongated, projecting over the outer chamber in the case of Szinkum mandarin. In the Florida seeding grapefruit the ridge of entrance is so short that its inner walls are nearly perpendicular, even in the closed stoma, and assume a more spread position in the open stoma. Another feature of interest, though common to both, is the extension of the cutinized tissue along the vertical walls of the guard cells down to the pores.

The under views (FIGS. 1, *C*, and 1, *F*) show clearly that the size of the pore, *p*, is approximately the same in the closed stomata of both species.

The main differences in the two species are, then, in the size of the opening in the cuticle, and in the shape of the ridge of entrance, which bounds this opening. The opening is much larger in the grapefruit than in the mandarin, and the ridge of entrance has its inner walls more nearly perpendicular to the leaf surface. The bearing of these differences upon the resistance to citrus canker will now be considered.

Canker is caused by a bacterium which is motile in water, but entirely passive in air. It is believed that it cannot attack or even by its own activity traverse dry cutinized or waxy cell walls of *Citrus*. The outer walls in both of the species studied are cutinized and normally dry, except when moistened by rain or dew. This is true also of the walls of the outer chambers of the stomata, which are likewise cutinized, as shown by the cross sections of both species studied (FIGS. 1, *B*, and 1, *E*). Granting the above to be true, the bacteria can only penetrate to the uncutinized cells of the air spaces inside the leaves in continuous films of water. In intact *Citrus* leaves these can only form through the stomatal openings.

If a *Citrus* leaf of either of the sorts studied is immersed in water and studied under the microscope, air bubbles are found in the stomatal openings. In cross sections these bubbles are seen to extend to the ridge of entrance of the stoma. Thus when a water film is formed over a *Citrus* leaf, this film is held outside of the stomatal openings by the ridges of entrance. With the swaying of the leaves in the wind and with changes in temperature of the air inside the leaves, there are variations in the pressure against the water films covering the stomata, such that there will be a tendency for the water covering the outer surface of the leaf to be drawn into the intercellular spaces.

It will require less pressure to drive the water film inward through a wide aperture with nearly parallel walls, such as form the sides of the outer portion of the outer chamber of the Florida seedling grapefruit, than will be required to drive a water film past a narrow opening, along receding walls, such as form the outer portion of the outer chamber of the stomata of the Szinkum mandarin. Therefore, assuming that a certain minimum pressure is necessary to drive water into the stomata of grapefruit and thus establish a passageway for the entrance of bacteria, then a much greater pressure will be required to accomplsh the same result in the case of Szinkum mandarin. Once water has passed the widest part of the outer chamber, it will then contract its air-water film as it approaches the pore, and surface tension will then accelerate instead of retard the process. When water has passed through the pore, it is then in contact with moist, un-

cutinized cell-walls, and if the bacteria penetrate by means of continuous water from the surface of the leaf to this point, they appear then to be able to persist and develop in most *Citrus* species, as shown by the inoculation experiments of Lee.

The differences in stomatal structure observed in the two kinds of *Citrus* here studied thus are of such a character as to account satisfactorily for the observed difference in their resistance to canker. Field observations and inoculation experiments with *Citrus* canker show that many of the other *Citrus* species and varieties possess resistance to canker of the same general character as that of the Szinkum mandarin but differing in degree. It may be that these other resistant and partially resistant sorts may show structural characters also resembling the Szinkum mandarin. Further study of stomatal structure and canker resistance among the Rutaceae is therefore desirable.

The writer wishes to express his thanks to Dr. N. L. Britton and his associates at the New York. Botanical Garden, particularly Dr. A. B. Stout, for their kindness in furnishing laboratory facilities and valuable suggestions during this study.

SUMMARY

1. Szinkum mandarin, which is resistant to *Citrus* canker, and Florida seedling grapefruit, which is susceptible, are compared, and from a review of previous studies, their difference in resistance is believed to be due to a difference in the character of the stomata.

2. Both sorts have stomata of about the same size and type, differing mainly in the ridge of entrance, which is broad in the mandarin variety, overarching the outer chamber and forming a narrow external opening. In the grapefruit variety it is narrow, making the upper part of the walls of the outer chamber nearly parallel and affording a large opening.

3. These differences are such as to practically exclude water from the stomata of the mandarin, whereas it can more readily enter those of the grapefruit.

4. The exclusion of water is sufficient reason to account for the resistance of *Citrus* varieties to canker, since the canker bacteria are motile when in water but not when dry.

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